

# An Approach in Updating Plant Metabolomics in Roots to Tolerate Anaerobic Submergence Stress



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**Abstract** An overabundance of environmental extremities classically called abiotic stresses has been the integral part of plant's growth and development. Since then plants are also well adapted with its full genetic potential in two responses: susceptibility and resistance. These are coordinated with the expression of genes in up/down regulation according to genotypic plasticity at varying degrees as well as durations. Among the stressors most of those are perceived through root system of plants directly from soil like drought, salinity, metals and metalloids, pH variability, chemicals toxicity, hypoxia/anoxia etc. With the expression potential of gene(s) and its induction roots are also able to epigenetic regulation in tolerance of the stress factors where without interference of DNA sequence are also most important. Epigenetic regulation is also inheritable in nature but rather than any alteration of DNA sequence it involves the nuclear protein (histone) amendment as well as chemical modifications like methylation. In roots tissues certain conserved DNA sequences in chimeric manner in a precise and stringent regulation process tunes the responses to stresses that differs from rest of the flanking sequences. With the most modern–state-of art including high throughput sequencing at different platforms epigenetic regulation in roots genomics has reached a significant milestones to characterize stress. Thus, breeding with roots genomics now has set an alternative approach where world environmental climatic changes are ameliorated or minimize in crops to a significant extent. This chapter would encase various aspects of roots epigenetic responses to abiotic stresses in overall aspects of technology and its usefulness in crop sustenance.

## 1 Introduction

The phenotypes or morphological appearance of plants is the combinational results of a number of dynamic interaction of different molecules like nucleic acid, proteins, carbohydrates, organic acids, fat residues, phenolics and many others metabolites.

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These are all well in coordination plants' developmental status as well as environmental inputs like light, temperature, salts, water, air, humidity etc. This demands a precise phenotypic explanation, particularly, for genome analysis excluding transcriptome, proteomes and metabolomes also. However, this approach not necessarily means effectivity in understanding as well as characterizing the total plant biology. So, an integrated approach is still in demand where metabolic studies with sequences of many reactions may be the linking for genes expression and phenotypic countenance. Thus, the question of metabolomes comes relevant to context of whole system biology of plants. This also brings special is of especial significance to satisfy the objective how plant system is responding to environmental variations. As already understood that gene expression ensures the potential of plants responses to adjust the stress, the accumulation and types of metabolites represent the keys to those adverse conditions. With understanding of different and other omics in deciphering the system analysis how metabolomics would be useful in assessment of stress tolerance in equally important. The metabolic profiling under set of environmental variations would be better in correlation for molecular and physiological activities of plants even under controlled or ambient condition of habitat. Even those metabolic profiling could be used as markers for selection pressure for better rootstocks in breeding programme.

## **2 Metabolomics Approaches for Deciphering the Stress Tolerance Under Submergence**

Like other strategies covering genomics, proteomics, transcriptomics with high through put sequencing of genes, transcripts and peptides, metabolomics also approaches plants stress tolerance through study of metabolomes. In plant system, may be in a single cell, tissues, organs, however, in a specific growth or oncogenic state, metabolomics forwards the collection of biochemical reactions in a specific or collective paths. These could be more informative with plant's responses to a stressful environment with different level of accumulation of biometabolites. In *Sensu stricto* it is within the 1200 kD of low molecular weight chemical residues (reaction precursors, intermediates and products in a reaction cycle), either primary or even secondary also are come under consideration of metabolomics. Almost the cases those are included the processed gene products either in structural of enzymatic proteins for a biochemical cycle(s) and thereby directly or indirectly present the functionality or viability of the tissues. With other predominant or supplementary functions these compounds are integral or indispensable in sense of plant structure or biomass, cell wall residues and cytoplasmic constituents, signaling residues, antioxidants and other moieties in plant's resistance to stress.

Irrespective of taxonomical hierchy in plant kingdom it covers around 200,000–1000,000 chemical residues as metabolites under varying concentration are documented those are quite flexible in their chemical diversity and property. Still, the

proper purification and identification through presumed annotation may be the full proof because of complexities and amalgamation with major and minor residues. By metabolic profiling may be the option in a wider identification of compounds with their predicted conformation and following tally with reaction kinetics. Therefore, the metabolomics would be more lenient to follow the abiotic stress paraphernalia with multidisciplinary approaches where a number of essentials must be the mandate like proper designing of the experiment, calibration of a vigorous protocols for data harvesting, finery in chemical analysis, efficient work station for data analysis, data combination with other omics and finally the validation of plants biological explanation. In more development metabolic profiling and metabolic finger printing a huge or vast array of data on metabolites could be procured without any earlier supposition. So, with global metabolome expression of any plants under any stress bioinformatics tools may be the better option to corroborate the hypothesis/predicted data with metabolites accumulated in real. This may give the satisfactory results with higher level of accuracy as well as meticulousness. With modern state of art an initial analytical separation following detection and identification are the most predominant domain to start the metabolomics.

### **3 Submergence Stress: A Significant Scope for Metabolomics Study**

Flood occurrence in the form of water logging or submergence poses a significant vulnerability for survival, growth and productivity. According to FAO it covers at least 10% of the total cultivable land is flooding prone with recurrent loss of grain yield in rice (Manik et al. 2019). Submergence due to flooding is the resultant for induced hypoxia/anoxia for a prolong duration for root system. The inadequate O<sub>2</sub> concentration in capillary water within the rhizosphere creates the well-known anoxic stress with significant loss of energy yielding metabolism like ATP synthesis (Ruf et al. 2019). The major changes in metabolomes are consisted, however, not limited with cytosolic acidification (lower pH values) as a function of impeded H<sup>+</sup>/ATP ase activity. Even with the transient occurrence of submergence due to flash flood apart from growth and productivity, plants are characterized by expression of core-hypoxic responsive genes (Kuroha et al. 2019). The later is mostly focused with rearrangement of root specific metabolomes or metabolites distribution. A set(s) of gene and its expression is mostly targeted to anoxia/hypoxia responses which may come under categories of: alcoholic/lactate fermentation, interconversion of sucrose starch residues, stress induced metabolites for osmotic adjustment like compatible solutes, the hypoxia induced growth suppressing metabolites like ethylene/ABA/GA occurrence and their interplay, generation and lysis of oxygen/nitrogen moieties with oxidative redox (Fischer et al. 2020). Likewise, of those selected genes, alanine amino transferase is set as a reliable bio-marker with its hyper accumulation of alanine in rice roots under prolong submergence (Lothier et al. 2020). On the other hand, the

allocation of reduced carbons into sucrose exerts a flux in partitioning of starch and other polysaccharides in roots. The land races with quiescence (an adaptation to reserve the energy in expense of growth suppression under complete submergence) followers are more used with starch phosphorylase expression and activities. Rice genotypes with Sub1 possessing traits are more practiced with transformation of complex polysaccharides from simpler reducing/non reducing soluble sugars into roots, culm and leaf sheath (Morel et al. 2019). The oxygen deficiency in roots, particularly, under complete submergence makes it different for the bio-metabolome pattern in roots than shoots which has fairly revelation to the aerobic condition either directly from environment or photosynthetic oxygen. In comparison to other stress, submergence must be linked to a composite of stress where almost the environmental extremities are the concerned except high irradiance (Chevrier et al. 2020). Still, in fully grown or adult plants the tissues/organ specific metabolic differentiation is incomplete under submergence stress as its depth and duration of water stagnation may significantly vary. Accumulation of reserved carbohydrates as found in rice culm and leaf sheath is more common under stress along with few stress metabolites like  $\gamma$ -amino butyric acid (GABA), alanine, polyols (Ghatak et al. 2018). A gradual change in plant's low molecular weight organic acids like malic acid from TCA cycle pool may characterize submergence sensitivity. This is operated in homeostasis with amino acid depletion in roots to demark the hypoxia/anoxia under submergence stress. In sugar metabolomics under waterlogged rice a linear decline in few amino acids may suggests inhibition of protein synthesis in contiguous with sugar export from source (leaf sheath, culm) to sink (roots and other submerged tissues). It is more discriminating for the nitrogen metabolomes in submerged roots where nitrate assimilatory reactions remain more sensitive (Srivastava et al. 2019). The oxygen deprivation in roots would circumvent the impaired energy yielding metabolism where ATP dependent nitrate reduction is down regulated. Alternatively, roots may be more lenient to NAD(P)H dependent reduction with lesser sensitivity to hypoxia (León et al. 2020). Still, leaf sheath and culm are not much affected in nitrate reduction as compared to roots since exposed to air and more accessible to photosynthetic oxygen in mesophyll tissues. In waterlogged cotton, soybean other metabolites like ureides, glutamines are inhibited to translocate into leaves from roots (Lothier et al. 2020). The translocation efficiency under water logging is fully or partially dependent on metabolite exchange through root cell sap (in xylem/phloem conduits). This is illustrated with *Ricinus* where phloem sap does not vary with sugar concentration in roots but sap flow rate as well as flow area within the phloem sieve tube significantly found reduced (Shen et al. 2020). This is well consistent with other cereals like *Zea* where labelled carbon ( $^{14}\text{C}$ -sucrose) fed in leaves had not metabolized into TCA cycle intermediates in roots under anoxia. Contrarily, derivatives of glucose, however, non-metabolites moiety like  $^{14}\text{C}$ -deoxyglucose had the minimum rate in translocation to roots under submergence induced anoxia (Maranas 2017). Despite of these there found few enigmas while someone takes the study of submergence metabolomics in roots that how it connects with areal shoots, whether phloem loading and unloading is independent through flux of metabolites. Under submerged

roots there is a tend to increase the phloem sugar loads that may exert an feedback inhibition on sugar export and downstream metabolism in shoots.

## 4 Areas Under Coverage of Root Metabolomics Under Submergence Stress

Under submergence plants have to experience a number of other abiotic stressors those may change directly or indirectly on morphological, anatomical, physiological, biochemical and cellular activities. Semi aquatic species like rice, roots survival under submergence are complemented by two sets of phenomena: escape and quiescence strategies. Both of those are targeted to assure to cope up the oxygen deficits either in form of hypoxia or anoxia. In the escape strategy the gas exchange and its rate from plants areal shoot to environment is increased with distinguishable features like those of epinasty, elongation of leaf sheath and internodes etc. Contrarily, energy expense is lesser in escape strategies with restriction of vegetative growth under water regimes. (The high energy depending phenomena like protein synthesis, DNA replication, cell wall synthesis are, secondary growth process are well regulated under quiescence with an observable alteration of cellular respiration from aerobic to anerobic (da Veiga Moreira et al. 2015). In sustenance to those the submergence tolerant cultivars are induced to sustain oxidative phosphorylation and allied metabolisms with formation of special tissues like aerenchyma in roots. Additionally, a number of metabolic activities like gas filled film in air spaces in leaf mesophyll is other support to coordinate the gas diffusion under anerobic/hypoxic condition under submergence (Liang et al. 2020). Along with high through put metabolomics a huge number of data are processed from roots responding to stress, particularly, in cereal crops. Both Gel based/free systems in roots proteomics could support the types of metabolites produced in different tissues in roots through either labeling or non labeling to know flow of reaction under stressed condition (Huihui et al. 2020). Since water logging or flooding stress may couple other facets of abiotic stresses the commonness and similarities of metabolites flux would more flexible to identify the nature of compounds in roots even at sub cellular levels also. Identification of complex proteins and metabolites are often challenged by presence of high number and amount of proteases, oxidative enzymes, and phenolics in tissues (Hashim et al. 2020). Still, metabolomic techniques like TCA precipitation, GITC extraction, SDS lysis, phenol phase separation and others chromatograms are the most uses for study of metabolomes in roots for any plants. Metabolomics coupled with proteomics had also great advantages in detection and analysis of target protein in roots even under post submergence period dried soil (Yan et al. 2020a, b). This releases the data on hyper/hyporegulation of different target enzymes and their contribution in metabolic fluxes in common responses for saturated-dehydrated soil. More specifically, the organ specific secondary metabolites like a plethora of phenolics glycosides would be well in concern for submergence induced moisture deficit as well as oxidative revelation

**Table 1** Submergence induced metabolic fluxes and their changes in roots for different cereal crops

Crop species	Stresses	Nature of metabolites fluxes	References
Rice	Submergence	Ethylene, Glycolic acid, Glyoxalic acid	Wu et al. (2017)
Wheat	Hypoxia/anoxia	Polyamines, proline, Glycine betaine, Glutein	Yan et al. (2020a, b)
Barley	Low light, alkaline pH	Tri acetic acid, Citric acid, lactic acid, ethanol, glydine	Park et al. (2009)
Sorgham	Oxidative stress, ROS accumulation	TCA, electron transportproteins, Cyto chrome c, amino acids, nucleotides	Khan et al. 2015
Maize	Salinity, alkalinity, ROS	Bicarbonate metabolism, stress protein, Fermented metabolites, Fe-S proteins, Ferulic acid, Siderophore	Ashraf et al. (2018)
Oat	Soilmoisture deficits, ionic imbalances	Secondary metabolites, Cell cycle proteins, H <sup>+</sup> /ATP ase, DNA-nucleotides, chaperones	Akey and Morrison (1983)
Bajra	High irradiances, low temperature	Stress metabolites, jasmonic acid,phloem sapesidues, polysaccharides, inulin	Damame et al. (2017)

for tolerance species like cereal crops (Lobo et al. 2020). Even metabolomics coupled with analytical techniques for proteomics study has elucidated specific metabolites which have sparingly regulation in enzymatic cascade and also for post translational modifications for specific environmental extremities like low irradiance, anerobic exposure, ionic/metallic variation, pH sensitivity etc under submergence (Khan et al. 2020) (Table 1).

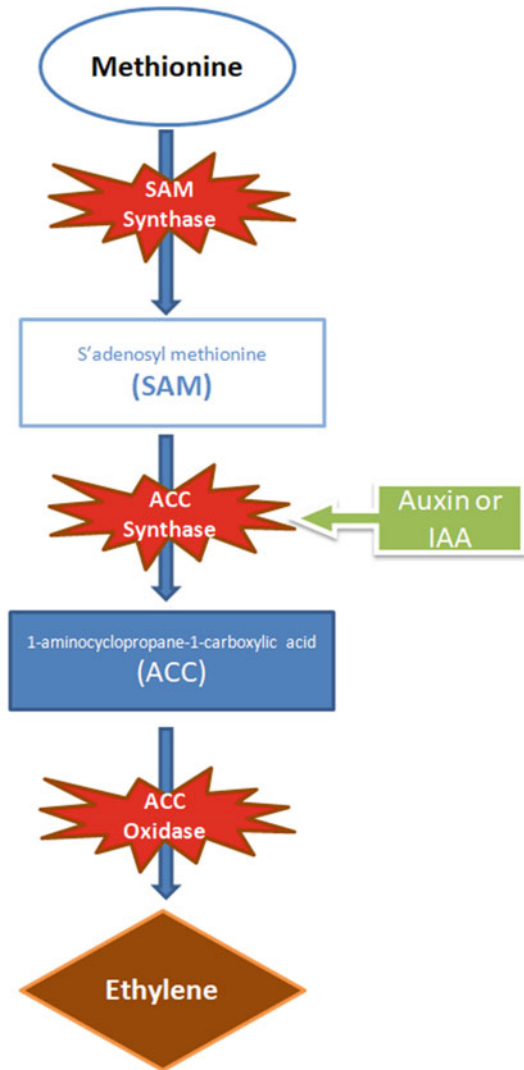
## 5 Compartmentalization of Metabolic Flux in Roots Under Submergence

The quite natural adaptation under submerged roots is radial oxygen movement from shoot to roots through functioning of aerenchyma (Pedersen et al. 2021). The metabolic flux that characterizes plants roots are the development of lysigenous cavities through programmed cell death that subsequent follows in lysis of the cortical cells. In roots of terrestrial species like *Zea*, *Triticum* etc. such an adaptation may not be availed by the plants specially under aerobic condition (Pegg et al. 2020). Still, those upland species may be induced with such lysigenous cavities under oxygen

deficit waterlogged condition. In typical semi aquatic cereal like *Oryza* such adaptation is readily also available under aerobic condition that may over expressed when plants are transferred to complete submergence of hypoxia/anoxia (Nakamura and Noguchi 2020). The metabolite compartmentalization is strictly sensed by plants for the special compounds like ethylene under submergence. Even with illustration with aquatic species ethylene biosynthesis and its involvement in special tissues may characterize the submergence tolerance (Chakraborty et al. 2021). In chemical reaction ethylene accumulation in rhizosphere and its diffusion through aerenchyma sets a special ecological niche for submergence sensitive plants that may differ a set of metabolites in cellular compartmentalization. Ethylene is produced from methionine residues in a more complex cycle compatible to polyamine biosynthesis in a competitive manner (Sauter et al. 2013). One intermediates like 1-amino-cyclopropane-1-carboxylic acid (ACC) is subsequently metabolized by ACC synthase (ACS) and ACC oxidase (ACO) to ethylene is the limiting factor for sensitivity to submergence for the species. The expression levels and polymorphisms of ACS1 and ACO5 increases to contribute, ethylene accumulation in the roots (Lee and Yoon 2018). The formation of aerenchyma is more compounded with the generation of reactive oxygen species (ROS) through a respiratory burst oxidase homolog H (RBOHH) which is in parallel overexpressed with ethylene (Fig. 1).

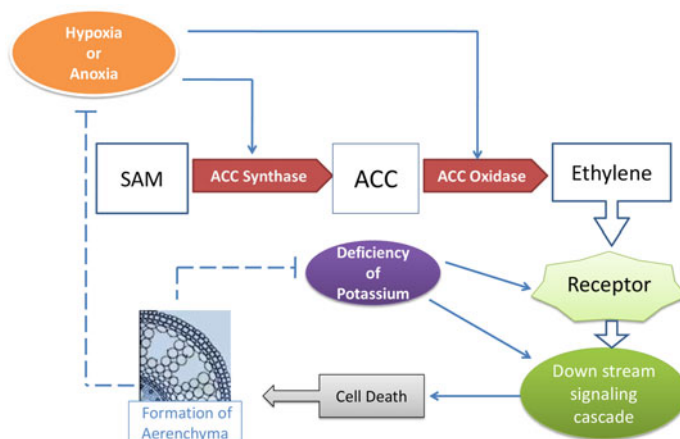
Another set of metabolite compartmentalization is limited in cortical tissues of rice roots where auxin signaling is distinctly perceived (Jun et al. 2011). A set of auxin response factors (ARFs) is regulated at specific auxin response element (AREs) in auxin induced genes where indole acetic acid binding proteins are most favored (Kim et al. 2020). In rice there recorded at least 25 ARF genes and 31 IAA genes which are highly variable in expression variable perception of hypoxia/anoxia (Wu and Yang 2020). IAA proteins are characterized with an most conserved sequence motif auxin-dependent proteolysis (Yan et al. 2020a, b). The correlation between ethylene formation and aerenchyma formation is well evident from auxin dependent mutation in roots gain of function (dominant-negative) *iaa13*. This mutant is characterized by a single amino acid substitution at the upstream (AUX/IAA domain II) of IAA13 protein (Yamauchi et al. 2019). Through the functional analysis of the *iaa13* mutant it comes in understanding for its involvement of inducible aerenchyma in rice roots. This is more established with the exercise of auxin inhibitor(s) where aerenchyma formation had been restricted under oxygen deficit or hypoxic condition of waterlogging (Yamauchi et al. 2020). This is also coordinated with ethylene biosynthetic gene activities to support more with the facts of auxin involved aerenchyma formation. In rice roots there proposed a mechanism where IAA-ethylene coordinated aerenchyma formation in relation to submergence tolerance. This also establishes that auxin is not only involved in constitutive aerenchyma formation but also tends to form same tissues in rice roots. This is also evident from *Arabidopsis* where ACC application would be a key factor for inhibition of lateral roots in accompany with increased auxin concentration in apical portion of roots. This is equally contradicted with ETHYLENE INSENSITIVE2 (EIN2) mutant for ethylene signaling gene under submergence of *Arabidopsis* roots (Negi et al. 2008). This is further noticed that auxin inhibitor simultaneously down regulates the expression of ACS1 and ACO

**Fig. 1** Possible mechanism of auxin regulated ethylene biosynthesis



gene activities under stagnant water logging condition. This is experimentally proved in maize roots also where application of auxin also induces the lateral roots formation with ethylene hyper expression, however, irrespective of anerobic condition (Yu et al. 2015). So, cellular compartmentalization of ethylene and auxin in lateral root formation would be another key to metabolite compartmentalization in submerged roots sensitivity, particularly, under anoxia (Fig. 2).





**Fig. 2** Possible pathway of ethylene biosynthesis and its down stream regulation in rice root under hypoxia or anoxia

## 6 Metabolic Fluxes of Reaction Oxygen Species in Roots Under Submergence

Flooding and heavy downpour could cause the stagnation of water in the form of waterlogging/submergence leading to soil compaction and/or erosion, inundation, reduced oxygen tension or hypoxia/anoxia and finally plants damages. Stress hormones predominantly ethylene, ABA are the factors for induction of genes under inundation as well as perceive signaling form submerged soil to plant roots insides (Voeselek and Bailey-Serres 2015). In roots meristem and cortical cells the accumulation of ethylene can stimulate the endoplasmic reticulum (ER) sited transmembrane protein named ethylene insensitive transmembrane protein 2 (EIN-2) which in downstream also induced a set of transcription factors Ethylene insensitive 3 (EIN3). The later one the most important for the ethylene responses element (ERS) to activate those genes induced by the ethylene (Yu et al. 2017). Under anoxia ethylene can control a number of other growth regulators like GA, ABA etc. for cellular modification of quiescence or escape strategies under in survival strategies of plants. Herein the role of cellular redox would be another module in reaction with root cells for elongating and adventitious roots through cell wall lysis. This is as comparable to the programmed cell death as commonly available in rice, maize like cereal roots tissues (Basu et al. 2020). About the source, types and function of ROS to modulate the cellular redox, it is the hydrogen peroxide ( $H_2O_2$ ) that function as a secondary messenger to ensure peroxidation reactions in membrane lipid lysis of endodermal layers of roots.

In a fine orchestration  $H_2O_2$  can induce the ROS paths with a number of variants like super oxide anion ( $O_2^-$ ), hydroxyl radical ( $OH^\cdot$ ), hydroxonium ions ( $OH_2^+$ ), singlet oxygen ( $^1O_2$ ) etc. In fact, rice roots are well adapted to anerobic condition

either by anaerobic respiration or alcoholic fermentation where these ROS are realized as byproducts (Sun et al. 2020). A well-known enzymatic cascade on different cellular organelle or non-cellular space that starts with respiratory burst oxidase homologue (RBOH) commonly NADP(H) oxidase is involved in ROS generation (Hong et al. 2020). In plants there recorded at least ten such genes in a multigene family to accommodate load of ROS generation as in *Arabidopsis*. The kinetics of different ROS are quite variable according to their chemical stability through the tissues when developed with anaerobic stress.

On downstream development of  $O_2^-$  in roots are well sensitized with super oxide dismutase (SOD) into  $H_2O_2$  (Saha et al. 2020). The later one being soluble ROS but more than a free radical can stimulate the cellular responses in two ways: anti-oxidation by peroxidase and elicitation of some other enzymatic reactions. Likewise,  $H_2O_2$  in turn can stimulate few other ethylene response factors (ERFs), alcohol dehydrogenase (ADH) like anaerobic proteins in roots under submergence. In a wider metabolic sense plant RBOHs are well characterized with other developmental processes where  $Ca^{+2}$  is most crucial. RBOHs could also moderate the  $Ca^{+2}$  efflux inside the cells which also bears relevance with adventitious root development (Demidchik et al. 2018). This is thoroughly studied in *Arabidopsis* where a homologue (*At* RBOHc) has been cloned with gentle NaCl treatment for development of lateral roots. Interestingly, those roots are good sensitive to other stresses like pathogenic or elicitation by symbiotic association (Sakuraba et al. 2015). With other variants in *Arabidopsis* like *At* RBOHd, *At* RBOHf is well coordinated in expression with transcript level under minimum salt differences in the growth media. More so, *At* RBOHd is reported with ABA signaling network for regulation of stomatal guard cells in a systematic response with other elicitation. As for e.g. signaling transduction for wound and biotic invasion, irradiances, heat and cold shock, abundances of salt and metals are the regular entities to exercise the ROS involvement and its consequent metabolism under roots in regulation of stress sensitivity (Luo et al. 2021). Undoubtedly, *At* RBOHd and its homologues are involved in stress perception to anoxia/hypoxia in roots but not much established in any direct relationship with ethylene metabolism. In earlier reports ROS like  $H_2O_2$  was found to be reduced in *ein 2-5* as well as *rbohD-ko* mutant when subjected to hypoxia stress. The major hypoxia induced genes like *Aldh* in rice was down regulated in expression in such *rbohD-ko* mutant (Kim et al. 2019). So, there are ample scope to further study for the interactive session of ethylene and  $H_2O_2$  in roots not only for submergence tolerance but also other responses like seedling root growth, pigmentation as well as anoxia gene functioning.

## 7 Metabolomics in Roots for Re-Oxygenation Phenomena on Post Submergence

While flooding plants are partially or fully inundated but plants are exposed to high oxygen tension as but water level subsides. This creates another environment of high oxygen concentration coupled with strong irradiance (Striker 2012). The achlorophyllous tissues in leaves and culm under hypoxia are more sensitive to oxidative stress that sets the secondary impact on submergence sensitivity. The roots are more aerated along with loss of membrane permeability for  $K^+$  and other osmolytes. The water deficit in root tissues may turn over to hyper turgidity and metabolically can not complete with ATP generation through non/poor functioning of oxidative energy metabolism (Rachmawati et al. 2019). At metabolic level for re oxygenation is evident with lipoxygenase activities in substantial accumulation of malondialdehyde content. A fall in compatible solute biosynthesis in roots leads to reduced permanent wilting percentage and finally ensures dehydration (Ayala et al. 2014). In reference to rice the major submergence tolerance regulator SubA, an ethylene response factor (ERFs) imparts the tolerance to oxidative and dehydration factors making submergence a multiple or composite stress. A number of ERFs are cloned from both rice and *Arabidopsis* where post submergence induced re-oxygenation are encountered with metabolic residues like ABA (ref). In fact, in rice roots re-oxygenation induces several motifs in ABA response element (ABRE) to bind with APETALA like factors (Saha et al. 2021). In a synchronized regulation of GAREs by respective factors, mostly bZip classes of proteins roots are maximized in oxidative stress tolerance by adopting quiescence strategies. For the later roots could regulate sugar utilization in aerobic respiratory flux by activation of genes like

Although the genetic mechanism of submergence survival for rice varieties containing the SUB1A gene has been elucidated, the downstream metabolic effects have not yet been evaluated. In this study, the metabolomes of *Oryza sativa* ssp. japonica cv. M202 and cv. M202(Sub1) were profiled using <sup>1</sup>H NMR spectroscopy to compare the metabolic effect of submergence stress and recovery on rice in the presence or absence of SUB1A. Significant changes were observed in the NMR resonances of compounds in pathways important for carbohydrate metabolism. The presence of SUB1A in M202(Sub1) was correlated with suppression of carbohydrate metabolism in shoot tissue, consistent with the role of SUB1A in limiting starch catabolism to fuel elongation growth. The absence of SUB1A in M202 was correlated with greater consumption of sucrose stores and accumulation of amino acids that are synthesized from glycolysis intermediates and pyruvate. Under submergence conditions, alanine, a product of pyruvate metabolism, showed the largest difference between the two varieties, but elevated levels of glutamine, glutamate, leucine, isoleucine, threonine, and valine were also higher in M202 compared with the M202(Sub1) variety. The identification and characterization of alanyl glycine (AlaGly) in rice is also reported. After 3 days of submergence stress, AlaGly levels decreased significantly in both genotypes but did not recover within 1 day of desubmergence with the other metabolites evaluated. The influence of SUB1A on dynamic

changes in the metabolome during complete submergence provides new insights into the functional roles of a single gene in invoking a quiescence strategy that helps stabilize crop production in submergence-prone fields.

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